Characterisation of *Agaricus bisporus* Response Genes to *Verticillium fungicola* infection

A. Costa[1,2]; D. J. I. Thomas[1]; A. Bailey[2]; G. D. Foster[2]; M. P. Challen[*1]; P. R. Mills[1]

[1] *Warwick HRI, University of Warwick, Wellesbourne, Warwickshire CV35 9EF, UK*
[2] *School of Biological Sciences, University of Bristol, Bristol, BS 1UG, UK*
mike.challen@warwick.ac.uk

The mycoparasite *Verticillium fungicola* is a persistent threat to the cultivation of the mushroom *Agaricus bisporus*. Mushroom "dry bubble" is characterised by an undifferentiated mass of cells and can result in major crop losses. During the establishment of "dry bubble" substantial changes occur in the biochemistry and physiology of both partners. To enable new insights to be made into the molecular events underlying the disease, work is in progress to identify genes expressed during pathogen infection. Subtractive Suppressive Hybridisation (SSH) has enabled recovery of 65 expressed sequenced tags (ESTs) differentially expressed during infection. After database searches 27 of the genes were identified as most likely from *V. fungicola*, 25 from *A. bisporus* and 13 unknown. Bioinformatic analysis suggested that the response genes identified were involved in a range of biological functions that included stress, signalling, protein synthesis and cell wall structure and function.

Specific full-length genes will be recovered using cDNA library constructed from lesions of *A. bisporus* infected with *V. fungicola*, enabling silencing approaches to be used to further investigate the role of the identified genes in disease. An alternative higher-throughput method of gene function analysis, RNA interference (RNAi) using *A. bisporus* model genes (*URA3*, *CBX*), is also being developed. Silencing constructs expressing RNAi hairpin were transformed into *A. bisporus* using *Agrobacterium tumefaciens* and hygromycin resistance. Screening of the transformants by PCR confirmed integration of the silencing construct in 24 transformants. RT-PCR is being used to confirm transcription of the RNAi hairpin. Quantitative PCR will be used to analyse levels of target gene transcripts post RNAi transformation. The role of *A. bisporus* genes identified, in the infection process, will be determined through infection trails with *A. bisporus* silenced lines.

Genetics and Cellular Biology of Basidiomycetes-VI. A.G. Pisabarro and L. Ramírez (eds.)
 ISBN 84-9769-107-5